### REMARKS

The above amendments to the above-captioned application along with the following remarks are being submitted as a full and complete response to the Official Action dated May 13, 2005.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

### Status of the Claims

Claims 1-4 and 8-14 are under consideration in this application. Claims 1, 3-4 and 11-12 are being amended, as set forth above, in order to more particularly define and distinctly claim Applicants' invention, while claims 6 and 7 are being canceled without prejudice or disclaimer. Support for the claims may be found throughout the specification including, for example, on page 20, lines 5-28; page 21, lines 19-28; page 26, line 21 to page 27, line 8; and page 29, lines 13-18. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

## Formal Rejections

The Examiner rejected claims 1 - 4 and 6 - 14 under 35 U.S.C. § 112, second paragraph, as being vague and indefinite. The Examiner cited language in the claims which needed either correction or clarification. As outlined above, the claims are being amended to correct the various formal errors and to more particularly point out and distinctly claim the subject invention.

# Prior Art Rejections

The Examiner again rejected claims 1-4 and 6-9 under 35 U.S.C. § 102(b) on the grounds of being anticipated by Nyren et al (WO 98/13523).

In addition, the Examiner rejected claim 10 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of the article to Ishikawa et al (Human Immunology (1995) 42:315-318).

Further, the Examiner rejected claims 1-4, 6-9, 11, 12 and 14 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of Nyren et al (WO 98/28440).

Even more, the Examiner rejected claims 10 and 13 under 35 U.S.C. § 103(a) as being unpatentable over Nyren '523 in view of Nyren et al (WO 98/28440), and further in view of Ishikawa et al. These rejections have been carefully considered, but are again most respectfully traversed.

The present invention as now recited in claim 1 is directed to a method of analysis of DNA sequence, comprising the steps of: treating a substrate solution containing a nucleic acid substrate for a complementary strand extension reaction by degrading, using pyrophosphatase, pyrophosphoric acid contained in the substrate solution, and/or degrading, using apyrase, adenosine 5'-triphosphate contained in the substrate solution; removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the pretreating step; mixing the substrate solution with reaction solution that contains a DNA primer, a target nucleic acid and a reagent for the extension reaction on the DNA primer, after the step of removing or inactivating; conducting the extension reaction on the DNA primer hybridized to the target nucleic acid, the extension reaction consisting of a plurality of one base extensions, wherein the substrate solution is added to the reaction solution per each of the plurality of one base extensions; and detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step, wherein the substrate solution does not contain the DNA primer, the nucleic target acid and the reagent.

The present invention as recited in claim 3 is now directed to a method of analysis of DNA sequence, comprising steps of: adding pyrophosphatase and/or apyrase to one or more solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid or adenosine 5'-triphosphate, respectively, contained in the one or more solutions; removing or inactivating the pyrophosphates and/or the apyrase in the one or more solution after the step of degrading after the adding step; mixing the one or more solutions[[,]] with a reaction solution that contains a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer, after the step of removing or inactivating; and extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the one or more solutions as a plurality of one base extensions; and detecting pyrophosphoric acid generated during an extension reaction by chemiluminescence-reaction after the removing or inactivating step, wherein the one or more solutions does not contain the DNA primer, the target nucleic acid and the reagent.

In addition, according to claim 4, the present invention is directed to a method of analysis of DNA sequence comprising steps of: adding pyrophosphatase to one or more

solutions each containing a different deoxynucleotide, or an analogue of the deoxynucleotide and then thereby degrading pyrophosphoric acid contained in the one or more solutions; removing or inactivating the pyrophosphates in the one or more solutions after the step of degrading after the adding step; mixing the one or more solutions[[,]] with a reaction solution that contains a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer, after the step of removing or inactivating the pyrophosphatase; extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of the one or more solutions and converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5'-phosphosulfate and ATP sulfurylase; and detecting luminescence caused by chemiluminescence-reaction using the adenosine 5'-triphosphate, a luminescence-enzyme and a luminescence substrate after the removing or inactivating step, wherein the one or more solutions does not contain the DNA primer, the target nucleic acid and the reagent.

Further, as recited in claim 11, the present invention is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to each of a first solution containing deoxyadenosine 5'-a-thiotriphosphate, a second solution containing deoxythymidine 5'-triphosphate, a third solution containing deoxyguanosine 5'-triphosphate and a fourth solution containing deoxycytidine 5'-triphosphate, and then thereby degrading pyrophosphoric acid contained in each of the first, second, third and fourth solutions; a second step of removing or inactivating the pyrophosphatase in each of the first, second, third and fourth solutions after the first step; a third step of mixing at least one of the first, second, third and fourth solutions with a DNA primer, a target nucleic acid and a reagent for extension reaction of the DNA primer after the second step; a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and at least one of solutions obtained in the second step, converting pyrophosphoric acid generated during the extension reaction into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5' triphosphate, lusiferase and luciferin after the second step, wherein each of the solutions does not contain the DNA primer, the target nucleic acid and the reagent.

Even more, the present invention as recited in claim 12 is directed to a method of analysis of DNA sequence, comprising steps of: a first step of adding pyrophosphatase to a first solution containing deoxyadenosine 5'-α-thiotriphosphate, deoxythymidine 5'-triphosphate, deoxyguanosine 5'-triphosphate and deoxycytidine 5'-triphosphate, thereby

degrading the pyrophosphoric acid contained in the first solution; a second step of removing or inactivating the pyrophosphatase in the first solutions after the first step; a third step of mixing the first solution with a DNA primer, a target nucleic acid and a reagent for an extension reaction of the DNA primer after the second step, and a fourth step of extending the DNA primer hybridized to the target nucleic acid, by using DNA polymerase and a solution obtained in the second step, converting pyrophosphoric acid, generated during the extension reaction, into adenosine 5'-triphosphate in presence of adenosine 5' phosphosulfate and ATP sulfurylase; and a fifth step of detecting luminescence caused by chemiluminescence-reaction using the adenosine 5' triphosphate, lusiferase and luciferin after the second step, wherein the first solution does not contain the DNA primer, the target nucleic acid and the reagent.

Among the main features of the present invention as recited in at least the abovenoted claims, the invention is characterized in "treating a substrate solution containing a
nucleic acid substrate for complementary strand extension reaction with degrading, using
pyrophosphatase, pyrophosphoric acid contained in the substrate solution", "removing or
inactivating the pyrophosphatase and/or the apyrase in the solution after the step of
pretreating", "mixing the substrate solution with a reaction solution that contains a DNA
primer, a target nucleic acid and a reagent for extension reaction of the DNA primer, after the
step of removing or inactivating", "wherein the substrate solution does not contain the DNA
primer, the target acid and the reagent," as recited, for example, in claim 1. These features are
similarly recited in independent claims 3, 4, 11 and 12.

Applicants will point out that the four dNTPs that are applicable to the present invention generate PPi (pyrophosphoric acid) by thermal degradation or the like and become the largest causes of signal noise and that, depending on the company from which a reagent was purchased, the manufacturing methods used, the lot and storage conditions, etc., the amounts of PPi contained as impurities in the four dNTPs or analogs thereof differ (page 27, lines 11-18). In order to reduce the cause of noise brought by too much PPi (pyrophosphoric acid) that is generated by thermal degradation of the nucleic acid substrate (dNTPs), the invention incorporates "treating a substrate solution containing a nucleic acid substrate for complementary strand extension reaction with degrading, using pyrophosphatase, pyrophosphoric acid contained in the substrate solution", "wherein the substrate solution does not contain the DNA primer, the target acid and the reagent", all as recited in the claims. Here, pyrophosphatase breaks the phosphate bond which is exists not only in PPi, but also dNTP.

At the initial stage of treating using pyrophosphatase, the amount of dNTP is much more than the amount of PPi and the amount of degraded dNTP can be negligible. However, after the degradation of PPi, pyrophosphatase degrades only the dNTP. That is, pyrophosphatase will degrade too much dNTP, if it remains in the solution for a long time. This results in the loss of dNTP and low sensitivity in the detection. To solve this problem, the invention includes the characteristics of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating". This characteristic of the invention can reduce the cause of noise while avoiding excess dNTP degradation, and thereby allowing sensitive detection of the chemiluminescence reaction.

Applicants respectfully contend that none of the cited prior art references teaches or suggests the combination of steps or features as noted above, as recited for present the invention.

In contrast, regarding the rejection under 35 USC §102, Nyren et al. (PCT WO 98/13523) is only directed to removing ATP from reagent solution prior to addition to reaction mix (p.7, lines 18-20). In order to achieve this action, Nyren '523 discloses that the solution is contacted with immobilized enzyme that converts ATP into a product which is no longer a substrate for luciferase (p.7, lines 20-23). Nyren '523 does not disclose or suggest using pyrophosphatase for degrading PPi (pyrophosphoric acid), as does the method of the present invention. Therefore, Nyren '523 cannot, by itself, anticipate or render obvious each and every feature of the present invention as claimed.

Further, with respect to the rejection under 35 USC §103, Nyren et al. (PCT WO 98/13523) merely discloses that the immobilized enzyme may be removed prior to the chain extension/detection (p.7, lines 25-26). However, Applicants will point out that the target to be removed is apyrase degrading for ATP. Nyren '523 does not disclose or suggest "using pyrophosphatase for degrading PPi (pyrophosphoric acid), as claimed for the invention. Thus, Nyren '523 cannot, by itself, render obvious any step to reduce the cause of noise with avoiding surplus dNTP degradation and realizes sensitive detection for chemiluminescence reaction.

The secondary reference of Nyren '840 does not show or suggest any step of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating, such as that recited in the claims. Nyren '840 merely discloses that "any possible contamination of the reagents e.g. the NTP solutions, by PPi is undesirable and may readily be avoided by including a pyrophosphatase, preferably in <u>low amounts</u>, in the reagent solutions"

(see p. 19, lines 2 -6). However, as described above, pyrophosphatase breaks the phosphate bond not only in PPi, but also in dNTP. Thus, if the pyrophosphatase remains in the NTP solution even in low amounts, it will continually degrade the NTP, thus inevitably resulting in excessive degradation of NTP and low detection sensitivity. Further, to the extent that Nyren '840 describes adding a pyrophosphatase in low amounts in the reagent solutions, or even allowing a pyrophosphatase to remain in low amounts, this reference falls far short of disclosing or even suggesting any step of removing or inactivating the pyrophosphatase and/or the apyrase in the solution after the step of treating. Therefore, Nyren '840 cannot and does not provide any disclosure or suggestion to make up for the deficiencies of Nyren '523 such that their combination can show or suggest at least the above features of the present invention as claimed, nor the advantages achieved through those features of the present invention.

With respect to the tertiary reference to Ishikawa, this reference merely describes the use of primers for detecting a single base difference between A2 alleles and other HLA-A alleles, having one extra mismatch at the second position from its 3'-end (See Abstract). Ishikawa fails to provide any teaching or suggestion that would make up for the deficiencies in Nyren '523 or in Nyren '840, as described above, such that their combination could render the features of the present invention obvious. In other words, even if these three references were combined, that combination would still fall short of embodying all the claimed features of the present invention. Thus, the present invention as claimed cannot be rendered obvious in view of Nyren '523. Nyren '840 and Ishikawa.

### Conclusion

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art references upon which the rejections in the Office Action rely, Applicants respectfully contend that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of the above-captioned application, the Examiner is invited to contact the Applicants' undersigned representative at the address and phone number indicated below.

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August 12, 2005